

Claims:

1. An immunosensor system with reduced interference, comprising:  
a first immunosensor that generates a signal based on the formation of a sandwich between an immobilized antibody, a target analyte and a labeled antibody, wherein a portion of the signal arises from non-specific binding of the labeled antibody in the region of the first immunosensor, and  
a second immunosensor that acts as an immuno-reference sensor and generates a signal that is the same as or predictably related to the degree of non-specific binding which occurs in the region of the first immunosensor, and has an immunocomplex between an immobilized antibody and an endogenous or exogenous protein that is in the sample and that is not the target analyte.
2. The immunosensor system of claim 1, wherein the first and second immunosensors are electrochemical sensors.
3. The immunosensor system of claim 1, wherein the first and second immunosensors are amperometric electrochemical sensors.
4. The immunosensor system of claim 1, wherein the first and second immunosensors are selected from the group, potentiometric sensors, field effect transistor sensors, conductimetric sensors, optical sensors, evanescent wave sensors, optical wave guides, thermometric sensors and acoustic wave sensors.
5. The immunosensor system of claim 1, wherein the system is in a disposable cartridge for measuring analytes in a sample.
6. The immunosensor system of claim 1, wherein the target analyte is selected from the group consisting of troponin I, troponin T, creatine kinase MB, procalcitonin, hCG, NTproBNP, proBNP, BNP and myoglobin, in a blood sample.

7. The immunosensor system of claim 5, wherein the immobilized antibody in the second immunosensor is to a plasma protein.
8. The immunosensor system of claim 5, wherein the immobilized antibody in the second immunosensor is to a protein selected from the group consisting of human serum albumin (HAS), bovine serum albumin (BSA), fibrinogen and IgG fc region.
9. The immunosensor system of claim 5, wherein the endogenous or exogenous protein in the sample is present at a concentration sufficient to bind more than 50% of the available immobilized antibody on the second immunosensor within about 100 seconds of the sample contacting the immunosensor system.
10. The immunosensor system of claim 5, wherein the immobilized antibody in the second immunosensor has an affinity constant of about  $1 \times 10(7)$  to  $1 \times 10(15)$ .
11. The immunosensor system of claim 1, wherein both antibodies are immobilized on microparticles of diameter in the range 0.01-5.0  $\mu\text{m}$ .
12. The immunosensor system of claim 1, wherein the endogenous or exogenous protein is present in a blood sample at a concentration of at least three orders of magnitude above the affinity constant of the antibody in the second immunosensor.
13. A method for assaying a target analyte while reducing interference in an immunosensor system, comprising:  
contacting a sample containing a target analyte plus an endogenous

or exogenous protein, which is not the target analyte, with an immunosensor system comprising

a first immunosensor that generates a signal based on the formation of a sandwich between an immobilized antibody, a target analyte and a labeled antibody, wherein a portion of the signal arises from non-specific binding of the labeled antibody in the region of the first immunosensor,

a second immunosensor that acts as an immuno-reference sensor and generates a signal that is the same as or predictably related to the degree of non-specific binding which occurs in the region of the first immunosensor, and has an immuno complex between an immobilized antibody and said endogenous or exogenous protein,

washing said first and second immunosensors with a wash fluid, and using the signal from the first immunosensor and the signal from the second immunosensor to determine a corrected analyte concentration in the sample.

14. The method of claim 13, wherein the first and second immunosensors are electrochemical sensors.

15. The method of claim 13, wherein the first and second immunosensors are amperometric electrochemical sensors.

16. The method of claim 13, wherein the first and second immunosensors are selected from the group, potentiometric sensors, field effect transistor sensors, conductimetric sensors, optical sensors, evanescent wave sensors, optical wave guides, thermometric sensors and acoustic wave sensors.

17. The method of claim 13, wherein the system is in a disposable cartridge for measuring analytes in a sample.

18. The method of claim 13, wherein the target analyte is selected from the group consisting of troponin I, troponin T, creatine kinase MB, procalcitonin, hCG, NTproBNP, proBNP, BNP and myoglobin.

19. The method of claim 13, wherein the immobilized antibody in the second immunosensor is to a plasma protein.

20. The method of claim 13, wherein the immobilized antibody in the second immunosensor is to a protein selected from the group consisting of human serum albumin (HAS), bovine serum albumin (BSA), fibrinogen and IgG fc region.

21. The method of claim 13, wherein the endogenous or exogenous protein in the sample is present at a concentration sufficient to bind more than 50% of the available immobilized antibody on the second immunosensor within about 100 seconds of the sample contacting the immunosensor system.

22. The method of claim 13, wherein the immobilized antibody in the second immunosensor has an affinity constant of about  $1 \times 10^7$  to  $1 \times 10^{15}$ .

23. The method of claim 13, wherein the endogenous protein is HAS in a concentration of about 100 ng/ml or more.

24. The method of claim 13, wherein the endogenous or exogenous protein is present in a blood sample at a concentration of at least three orders of magnitude above the affinity constant of the antibody in the second immunosensor.

25. The method of claim 13, wherein both antibodies are immobilized on microparticles of diameter in the range 0.01-5.0  $\mu\text{m}$ .

26. The method of claim 25, wherein the microparticle is a carboxylate modified polystyrene bead.
27. The method of claim 13, wherein the signals from the first and second immunosensors are used to monitor the washing efficiency.
28. The method of claim 13, wherein the signals from the first and second immunosensors are used to detect anomalous sample conditions including improperly anti-coagulated samples.
29. An immunoassay device for measuring an analyte in blood while reducing interference associated with buffy coat-formed elements, comprising:  
providing a conduit for receiving a whole-blood sample in an immunoassay device, said conduit containing a salt reagent and means for treating the sample sufficient to increase the ionic strength of the sample and thereby reduce interference from the buffy coat when the sample contacts the immunosensor.
30. The device of claim 29, which is a disposable cartridge for measuring analytes in whole blood.
31. The device of claim 30, wherein the reagent comprises enough salt to increase the sodium concentration of the sample to at least 200 mM.
32. The device of claim 30, wherein the reagent contains a buffer and a sugar to promote rapid dissolution of the reagent into the sample.
33. The device of claim 32, wherein the reagent is a dry reagent

containing a mixture of sodium chloride, lactitol DEAE dextran and Tris buffer.

34. The device of claim 29, wherein the reagent comprises sodium chloride.

35. A method for measuring an analyte in blood in an immunoassay device while reducing interference associated with leukocytes, comprising:

adding a salt reagent to a whole-blood sample for an immunoassay device to increase the ionic strength of the sample and thereby reduce interference from the buffy coat when the sample contacts the immunosensor.

36. The method of claim 35, wherein the sample is used in a disposable cartridge for measuring analytes in whole blood.

37. The method of claim 36, wherein the reagent comprises enough salt to increase the sodium concentration of the sample to at least 200 mM.

38. The method of claim 36, wherein the reagent contains a buffer and a sugar to promote rapid dissolution of the reagent into the sample.

39. The method of claim 38, wherein the reagent is a dry reagent containing a mixture of sodium chloride, lactitol DEAE dextran and Tris buffer.

40. The method of claim 38, wherein the immunosensor system further comprises a bulk conductivity sensor and computation means for processing signals from the immunosensor and the conductivity sensor to correct for the hematocrit of the sample and determine the equivalent plasma analyte concentration.

41. An immunosensor system for blood with reduced interference, comprising:

an immunosensor for blood samples that generates a signal based on the formation of a sandwich between a first immobilized antibody to a target analyte, the target analyte, and a labeled antibody, wherein the sensing surface of said immunosensor contains a second immobilized antibody covering at least a portion thereof that forms an immunocomplex between an endogenous or exogenous protein that is in the sample and that is not the target analyte.

42. The immunosensor system of claim 41, wherein the first and second immunosensors are electrochemical sensors.

43. The immunosensor system of claim 41, wherein the first and second immunosensors are amperometric electrochemical sensors.

44. The immunosensor system of claim 41, wherein the first and second immunosensors are selected from the group, potentiometric sensors, field effect transistor sensors, conductimetric sensors, optical sensors, evanescent wave sensors, optical wave guides, thermometric sensors and acoustic wave sensors.

45. The immunosensor system of claim 41, which is in a disposable cartridge for measuring analytes in a sample.

46. The immunosensor system of claim 41, wherein the target analyte is selected from the group consisting of troponin I, troponin T, creatine kinase MB, procalcitonin, hCG, BNP, proBNP, NTproBNP and myoglobin.

47. The immunosensor system of claim 41, wherein the second immobilized

antibody is to a plasma protein.

48. The immunosensor system of claim 41, wherein the second immobilized antibody is to a protein selected from the group consisting of human serum albumin (HAS), bovine serum albumin (BSA), fibrinogen and IgG fc region.

49. The immunosensor system of claim 41, wherein the second immobilized antibody has an affinity constant of about  $1 \times 10^{-7}$  to about  $1 \times 10^{-15}$  M.

50. The immunosensor system of claim 41, wherein both antibodies are immobilized on microparticles of diameter in the range 0.01-5.0  $\mu\text{m}$ .

51. The immunosensor system of claim 41, wherein the endogenous or exogenous protein is present in a blood sample at a concentration of at least three orders of magnitude above the affinity constant of the second antibody.

52. The immunosensor system of claim 41, further comprising a bulk conductivity sensor and computation means for processing signals from the immunosensor and the conductivity sensor to correct for the hematocrit of the sample and determine the equivalent plasma analyte concentration.

53. The immunosensor system of claim 41, wherein the a whole-blood sample is treated to reduce interference associated with leukocytes by adding a salt reagent to increase the ionic strength of the sample to at least 200 mM sodium.

54. An immunoassay device for measuring an analyte in blood and correcting for the hematocrit of the sample to give an equivalent plasma analyte concentration, comprising:



providing a conduit for receiving a blood sample in an immunoassay device, said conduit containing an immunosensor and a bulk conductivity sensor,

providing computation means for processing signals from said sensors and determining the equivalent plasma analyte concentration.

55. The immunoassay device of claim 54, wherein the immunosensor is an electrochemical sensor.

56. The device of claim 54, wherein the cartridge contains a reference-immunosensor used to determine a corrected analyte concentration in the sample.

57. A method of performing an immunoassay for an analyte in blood and correcting for the hematocrit of the sample, comprising:

- (a) contacting an immunosensor with a blood sample,
- (b) contacting said blood sample with a bulk conductivity sensor and measuring its resistance,
- (c) contacting the immunosensor and conductivity sensor with an aqueous solution having known conductivity and containing reagents sufficient to generate a detectable product related to the amount of analyte bound to the immunosensor,
- (d) measuring the signal at the immunosensor generated by said product,
- (e) converting by means of an algorithm the signal from the immunosensor to an analyte concentration;
- (f) calculating the hematocrit value of the blood sample from the measured resistance of the blood sample, and
- (g) correcting the calculated analyte concentration for the hematocrit of the sample.

58. The method of claim 57, wherein the immunosensor is an electrochemical sensor.
59. The method of claim 57, in which the resistance of the aqueous solution in (c) is measured at the conductivity sensor.
60. The method of claim 59, in which the hematocrit value in (f) is further calculated using the measured aqueous solution resistance and the known conductivity of the aqueous solution.
61. The method of claim 57, wherein the sample contains a salt reagent added to increase the ionic strength and thereby reduce interference from leucocytes.
62. The method of claim 57, wherein the sample is used in a disposable cartridge for measuring analytes in whole blood.
63. The method of claim 61, wherein the reagent comprises enough salt to increase the sodium concentration of the sample to at least 200 mM.
64. The method of claim 62, wherein the cartridge contains a reference-immunosensor used to determine a corrected analyte concentration in the sample.
65. An amperometric immunosensor, comprising: an electrochemical sensing surface, for a blood sample, having a porous polyvinylalcohol layer patterned to cover at least a portion of the surface such that said layer attenuates background current from a blood sample to at least half the background current obtained in the absence of the layer.

66. The immunosensor of claim 65, wherein an antibody is attached to said porous polyvinyl alcohol layer on latex particles.
67. The immunosensor of claim 65, wherein the polyvinyl alcohol layer is patterned with a stilbizonium crosslinking agent and has a thickness 0.1 to 10  $\mu\text{m}$ .
68. The immunosensor of claim 65, wherein the polyvinyl alcohol layer is patterned with a stilbizonium crosslinking agent and has a thickness of about 0.6  $\mu\text{m}$ .